This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problems Mailbox.

Republic of France
National Institute
of Industrial Property
PARIS

Publication Nº 2 774 905
(To be used only for reproduction requests)
National Registration Nº 98 02 152
Int. Cl.: A 61 7/48

PATENT APPLICATION A1

Date of Filing:	17.02.1998	Applicant:	CODIF INTERNATIONAL S.A., Limited Company – FR
Priority:		Inventor(s):	GEDOIN ANTOINE, VALLEE ROMUALD and ARS HELENE
Date on which this patent was made available to the public:	20.08.99 Bulletin 99/33		
List of the documents cited in the preliminary search report:	Please refer to the end of this document.	Holders:	
		Attorney(s):	OFFICE OF LE GUEN ET MAILLET

USE OF A COSMETIC FORMULATION CONTAINING AT LEAST ONE SUBSTANCE THAT CONTAINS A PYRONE GROUP.

The present invention concerns the use of a cosmetic formulations containing at least one substance that includes a pyrone group.

Said utilization is such that it stimulates lipolysis.

Pursuant to one example, said substance is made up of a collagen acid or a member of the coumarin family or even consisting of a compound of the flavenol group of compounds.

The present invention concerns the utilization of a cosmetic composition containing at least one substance that includes a pyrone group.

The cosmetic compositions containing pyrone group substances, such as mangostine, are notably used to protect the skin from the sun because of their good absorption of ultraviolet radiation. These compositions are also known for their skin clearing action and in controlling inflammation following exposure to the sun. Such compositions are likewise used in the treatment of ulcerating diseases involving the pylorus, for example.

We refer to the Japanese patent documents JP-A-9-087 155, JP-A-6 0655 042 or JP-A-8 208 501 for examples of such uses.

The purpose of this invention if to propose a new use of a cosmetic composition containing at least one substance that include a pyrone group.

For this purpose, said utilization is such that it consists of stimulating lipolysis.

Pursuant to another characteristic of the invention, said utilization consists of activating, within the adipocytes, phosphorylation of an enzyme of the lipase for breakdown of the triglycerides into fatty acids.

Pursuant to another characteristic of the invention, said utilization consists of increasing the concentration of cyclic adenosine monophosphate in the adipocytes.

In accordance with another characteristic of the invention, said substance that contains a pyrone group is made up of a compound from the xanthone family of compounds, for example, xanthone or mangostine.

In this latter case, said mangostine is preferably extracted from a tree of the species of *Garcinia mangostana*, advantageously from the sap of said tree and or from the husk and the dried peduncles of its fruit.

In accordance with another example of the invention, said substance that includes the pyrone group is made up of a cojic acid.

In accordance with another example of the invention, said substance that includes the pyrone group is made up of a compound from the coumarin family of compounds, for example, coumarin.

According to another example of the invention, said substance that includes a pyrone group is made up of a compound from the flavenol family of compounds, for example, kaempferol.

The characteristics of the invention mentioned above, as well as others, will become clearer when reading the following description of several examples of realization.

The cosmetic composition that is used in the example of the invention includes mangostine, a substance containing a pyrone group and belonging to the xanthone family of compounds. The structural formula of mangostine is:

[Figure]

This substance is extracted from a tree known by the common name of mangostine whose species is identified by the designation *Garcinia mongostano*. That tree is found in certain tropical countries and in the region of Southeast Asia.

Preferentially, the substance is extracted from the hull and the dried peduncles of the fruit that is produced by the tree; said fruit is known by the common name of

"mangostine." However, it is also possible to extract the substance from the pulp of the fruit, or even from other parts of the tree, such as the bark or the dried sap.

Then the cut mixture is plunged into a solvent that permits the extraction of the mangostine from the mixture. In this example, the concentration of the described mixture is 10 g per 100 ml of solvent. By way of suggestion, for example, ethanol or acetone can be used as the solvent, or even any other organic solvent in which the mangostine can be dissolved.

The solution comprised of the chopped mixture and the solvent are stirred in order to dissolve the mangostine in said solvent. Then it is subjected to a clarifying filtration of the solution, the filtrated being made up of the solvent and the mangostine dissolved in it. As concerns the filter cake, it is composed of the chopped mixture from which the mangostine has been removed by the use of said solvent.

Preferably the filter cake would be washed with the solvent and the entirety would be mixed in such a was as to extract the residual mangostine from the filter cake. After the filtering phase as described in the foregoing, a second filtrate is collected in a manner similar to the foregoing that is composed of the solvent and the dissolved, residual mangostine in the solvent.

In this preferred example, said second filtrate containing the residual mangostine is then added to the initial filtrate obtained in such a way that a solution of solvent rich in dissolved mangostine is obtained.

The 1 ml of dipropylene glycol is added to the two filtrates described above, then the solvent containing the initially extracted mangostine and the residual mangostine in a dissolved state is eliminated by evaporation; thus, the mangostine is further concentrated.

In a general way, it is known that in the human adipocyte, an enzyme known as adenosine monophosphate 3,5-cyclic phosphodiesterase (cAMP-PDE class III, also called PDE in the following) catalyzes the transformation of cyclic adenosine monophosphate (cAMP) to 5-adenosine monophosphate and thus diminishes the intracellular concentration of cAMP. Likewise, it is known that an increase in the concentration of cAMP in the adipocytes has the effect of activating an lipase enzyme by phosphorylation of the enzyme and the enzyme then catalyzes the lipolysis reaction of the triglycerides into fatty acids and to glycerol.

Substances are known of that are capable of inhibiting to some degree the PDE in such a way as to increase the concentration of cAMP in the adipocytes and, as a consequence, bring about stimulation of lipolysis. Theophyline is one example of a PDE inhibitor in beef based on experiments done on bovine brain (see J Biol Chem, 1962, 237. 1224). Milrinone is another example of specific inhibitor of class III PDE.

In an initial experiment, the attempt was made to demonstrate a possible inhibitor effect relevant to PDE of the base mangostine product obtained using said example (called the M product in the following) in comparison to a theophyline product base reference.

FIRST *IN VITRO* EXPERIMENT

Several M product which were dissolved in dimethylsulfoxide (DMSO) and diluted to different concentrations in such a way that the final DMSO concentration was 5% (v/v) for each composition.

As a reference test, a theophyline-based product was prepared dissolved in ultra-pure water at 70 °C in such a way that the concentration of theophyline was 10 mM.

Concerning the PDE enzyme, it was obtained starting with a complex of bovine heart containing the PDE; said complex likewise contained calmodulin and some calcium. The enzyme test solution was prepared at 50 µg/ml in a test tube. Then the enzyme was incubated in the presence of its substrate consisting of tritiated cAMP (^{3}H cAMP).

This incubation was done in the absence of any other product (control test), in the presence of theophyline present at a concentration of 10 m/M (reference test) and in the presence of said M product which was diluted in order to proved concentrations (v/v) of 0.1, 0.5, 1.2 and 5 %.

The principle inherent in the dosage done by means of PDE is based on the use of a support that is made up of yttrium silicate beads and zinc sulfate. Under these ideal conditions, the linear nucleotides (AMP) link to the support; this is not the case for the cyclic nucleotides (cAMP). The ^{3}H 5-AMP formed by the reaction due to the PDE enzyme excites the scintillating substance present in the beads and generates a signal when it attaches to the beads.

After incubation the quantity of ^{3}H 5-AMP is measured by means of a beta scintillation counter. The results are shown in Table I and in graphs 1 and 2, below, in counts per minute (cpm) and in rate of activity of the PDE with respect to that of a test control (without PDE) for said test controls, reference tests, and diluted M products. Also shown in the tables are the results obtained on the one hand in the absence of PDE and on the other hand, in the present of PDE and dipropylene glycol (DPG) and DMSO, both at a concentration of 5% (v/v).

Table I

Products Tested	cpm	Number of Measurements	cpm Control	PDE Activity Rate	Rate of Inhibition
Control	2.316	3	0	0 %	
PDE control	27.685	3	25.368	100 %	
Theophyline 10 mM	2.714	2	397	2 %	98 %
PDE control + DMSO 5%	17.175	3	14.341	100 %	
+ DPO 5 % + DMSO 5 %	16.116	3	13 799	93 %	7%
M product 2 % + DMSO 5 %	569	3	-1 848	-12 %	112 %
M product 2 % + DMSO 5 %	587	3	- 1 730	- 12 %	112 %
M product 1 % + DMSO 5 %	459	3	- 1 857	- 13 %	113 %
M product 0.5 % + DMSO 5 %	1 123	3	- 1 197	- 8 %	108 %

M product 0.1 % + DMSO 5 %	1 518	3	- 798	- 5 %	105 %

Figure 1

Glossary

temoine	control / blank
controle PDE	Phosphodiesterase control
THEO	theophyline
DMSO	dimethylsulfoxide

Figure 2

Glossary

controle PDE (%)	Phosphodiesterase control (%)
controle PDE	Phosphodiesterase control

In conclusion, one observes immediately that if the 5 % DMSO significantly reduces the activity of the PDE (by about 40 %), the DPG added to the 5 % DMSO does not notably alter the PDE activity measured in the presence of DMSO.

It must be noted that the M product inhibits totally the PDE activity and this occurs in all of the concentrations tested. Moreover, the cpm [counts per minute] values measured are less than those observed for the control tests; that is, they are lower than those measured without PDE.

SECOND *IN VITRO* EXPERIMENT

In a second experiment the attempt was made to demonstrate a possible inhibitory effect versus PDE of the M product and for four other products in accordance with the invention containing a pyrone group and compared to a reference product that is composed of a milrinone compound.

One of the four other products tested is composed of a xanthone compound and has the following structural formula:

[Figure]

Another product tested, is made up of the cojic acid molecule and has the following structural formula:

[Figure]

Another product tested is coumarin, a compound belonging to the coumarin family of compounds and which has the following structural formula:

[Figure]

A final product tested is kaempferol, a compound belonging to the flavenols and having the following structural formula:

[Figure]

This experiment was conducted using class III PDE originating from human platelets that were incubated for 10 minutes at 30 °C in a substrate consisting of ^{3}H cAMP at a concentration of 0.1 µM.

A liquid scintillation method was used for the detection by counting the product formed (^{3}H 5-AMP) by the enzymatic reaction and several concentrations of each product described in the invention were tested for the purpose of determining the inhibition acting upon the PDE of human origin.

The results obtained regarding the human PDE are shown in Table II, below, in terms of the rate of inhibition of the activity of the PDE.

Products Tested	Concentrations Tested	PDE Inhibition Rate / One blank test without product
Reference milrinone	0.8 µM	50 %
M product	0.02 % (w/v)	98 %
M product	0.2 %	137%
M product	0.5 %	176 %
Xanthone	0.0005 mg/ml	12 %
Xanthone	0.005 mg/ml	31 %
Xanthone	0.05 mg/ml	55 %
cojic acid	0.5 mg/ml	27 %
Coumarin	0.005 mg/ml	< 10 %
Coumarin	0.05 mg/ml	19 %
Coumarin	0.5 mg/ml	81 %
Kaempferol	0.1 mg/ml	83 %

As shown in the table, the M product tested at 0.2 % (w/v) totally inhibits the reaction, where the xanthone tested at 0.05 % (or 0.005 %) inhibits 55 %, cojic acid tested at 0.5 mg/ml (or 0.05 %) inhibits 27 %, the coumarin tested at the same concentration inhibits 81 % and the kaempferol tested at 0.1 mg/ml inhibits 83 %.

In conclusion, it can be deduced that the pyrone group [figure] that is present both in the xanthones (such as M product and xanthone), in cojic acid, in coumarin (and other molecules of the coumarin group of compounds that are derived from coumarin) and in kaempferol (and other molecules of the flavenol group of compounds such as quercetol), has the effect of sensitively inhibiting the activity of class III PDE.

THIRD *IN VITRO* EXPERIMENT

In this third experiment the attempt was made to demonstrate a possible effect of the M product described in the invention in comparison to a reference product composed of the theophyline molecule, on the activation by phosphorylation of an adipocellular lipase enzyme. This enzyme is specific to the lipase mechanism and in this experiment involved the major lipase enzyme known by the name of HSL (hormone-stimulated lipase, called hormone-dependant lipase in the following).

Adipocytes isolated from an abdominal procedures in a 34 year old woman were used in this experiment and the respective techniques known as immunoprecipitation and Western blot were used for the purpose of quantifying the phosphorylation of the HSL enzyme under the effect of the products tested.

This experiment demonstrated that theophyline stimulated by a factor between 2 and 3 phosphorylation of the HSL enzyme, whereas M product stimulates said phosphorylation by a factor equating to 1.7.

The activating effect of the M product on phosphorylation of said enzyme that is responsible for lipolysis is thus confirmed.

FOURTH *IN VITRO* EXPERIMENT

In this fourth experiment the attempt was made to demonstrate a possible effect of the M product being tested on the activation by phosphorylation of said adipocellular lipase that is responsible for the breakdown of triglycerides into fatty acids and into glycerol.

The lipolysis reaction was followed by concentration of the liberation of fatty acids by the adipocytes in the presence of M product and in the presence of the three reference products (adrenaline, theophyline, and caffeine).

It is known that adrenaline stimulates lipolysis by acting in concert with the membrane receptors that are present on the adipocyte surface and that play a major role in the regulation of lipolysis (these receptors are called α, β or γ adrenergic). With respect to theophyline and caffeine, it is known that they favor hydrolysis of the triglycerides by inhibiting phosphodiesterase (PDE) which increases the intracellular concentration of cyclic AMP (cAMP).

Said lipolysis was followed in the presence of each of the products mentioned in the above by measurement of the liberation of lactate dehydrogenase (LDH), a cytosolic enzyme in the environment of incubated human adipocytes.

One began by isolating said adipocytes starting with fat tissue coming from surgical sources which was collected after abdominal procedures done on two female patients. One of the female patients, 55 years of age, underwent an initial set of the tests and the second female patient, 29 years of age, underwent a second set of the tests.

For the adipocyte dissociation medium (medium 1) a conventional medium known as MEM was used containing with phenol red and to which 50 IU/ml of penicillin, 50 µg/ml of streptomycin and 0.1 % (w/v) collagenase was added. And for the incubation medium for the dissociated adipocytes a medium 2 made up of the medium 1 to which 0.5 % (w/v) BSA (bovine serum albumin) was added.

The M product being tested was initially diluted to 50 % (v/v) either in ethanol or in DMSO, then said medium 2. Then the M product was tested at the final concentrations of 0.1 %, 0.5 %, and/or 1 % (v/v). The ethanol or DMSO concentration was maintained constant and equal to 5 % (v/v) for the first test and at 1 % for the second test. In addition, the "control [blank]" products were tested in parallel containing 0.5 % and 1 % (v/v) ethanol or DMSO.

Concerning the three reference products mentioned above, they were tested at 10^{-5} M and 10^{-4} M for adrenaline and 10^{-4} M and 10^{-3} M for theophyline and caffeine.

Each of the M products to be tested and each of the cited reference products were placed in contact for 2 hours at 37 °C with an appropriate number of cells.

For the purpose of evaluating cellular lysis at the concentration of said lactate dehydrogenase (LDH) the results were expressed in international milliunits (mIU) of LDH that is liberated into the adipocyte incubation medium.

In order to evaluate the respective lipolytic activity, the liberated fatty acids were dosed and the resulting values were expressed in nanomoles of fatty acids released into the incubation medium.

The results of the first assay are given in the following:

- adrenaline tested at 10^{-5} M and 10^{-4} M increases by a factor of 30 and 18, respectively, the liberation fatty acids;
- theophyline tested at 10^{-4} M and 10^{-3} M increases by a factor of 19 and 29, respectively, the liberation of fatty acids;
- caffeine tested at 10^{-4} M and 10^{-3} M increases by a factor of 19 and 29, respectively, the liberation of fatty acids;
- ethanol at 0.5 % (v/v) diminishes said liberation by 77 %;
- DMSO at 0.5 % (v/v) has no effect on said liberation;
- The M product being tested at 0.5 % (v/v) in ethanol or DMSO increases said liberation by a factor of 3.4;

In view of the results of this first assay, the M product tested at 0.5 % thus exhibits significant lipolytic activity.

The results of the second assay are given in the following:

- adrenaline tested at 10^{-5} M and 10^{-4} M increases by a factor of 9.1 and 8.5, respectively, the liberation fatty acids;
- theophyline tested at 10^{-4} M and 10^{-3} M increases by a factor of 7.8 and 8.4, respectively, the liberation of fatty acids;
- caffeine tested at 10^{-4} M and 10^{-3} M increases by a factor of 6.3 and 7, respectively, the liberation of fatty acids;
- ethanol at 1 % (v/v) had no effect on said liberation;
- DMSO at 1 % (v/v) increases said liberation by a factor of 2.9;
- The M product being tested at 0.1 % 0.5 % (v/v) in ethanol 1 % (v/v) has no effect on said liberation. On the other hand, M product tested at 1 % (v/v) increases said liberation by a factor of 1.5 %
- The M product tested ad 0.1 % and 0.5 % (v/v) in DMSO 1 % (v/v) has no effect on said liberation. On the other hand, M product tested at 1 % (v/v) increases said liberation by a factor of 2 with respect to the control products mentioned.

In view of the results of this second assay, the M product tested at 1 % (v/v) in ethanol or in DMSO exhibits significant lipolytic activity which confirms the results of the first assay.

Conclusion: At the time of the third experiment it was confirmed that the M product makes possible activation of phosphorylation of the adipocyte lipase. As this lipase is itself activated by cyclic adenosine monophosphate (cAMP) which plays the role of messenger for said phosphorylation and, as inhibition of PDE by said M product (as well as by the four other products that include the pyrone group; i.e., the second assay) it has precisely the effect of increasing the cAMP level, one can conclude that the products containing the pyrone group permit the increase of said levels and thus, stimulate lipolysis.

FIFTH *IN VITRO* EXPERIMENT

The attempt was made to determine in the framework of this experiment if an M product as described in the invention is capable of blocking the known inhibitor effect of neuropeptide Y (NPY) vis-à-vis lipolysis.

Human adipocytes were obtained after an abdominal procedure done on a 34 year old female patient. The adipocytes were then incubated for 2 hours at 37 °C in an incubation medium as described in the foregoing, in the absence of product for the first control test, in the presence of NPY at $7 * 10^{-7}$ M or a second reference test, and in the presence of NPY at $7 * 10^{-7}$ M and M product at 1 % (v/v) for a third control test of the effect of said M product.

After the two hours the quantity of liberated fatty acids into the respective incubation media was determined using the methods described in the foregoing. The results obtained are shown in the figure below.

Figure 3

Glossary

Liberation des acids gras	Liberation of fatty acids
(% de temoin)	(% of blank/control)
Témoin	Blank/Control

The figure shows that NPY inhibits by 45% the base liberation of adipocyte fatty acids. In addition, it shows that M product at 1 % not only cancels the inhibitor effect of NPY with respect to fatty acids, but even liberates almost as much fatty acid as if it were alone. The lipolytic effect due to M product is thus significantly preserved to the deficit of the inhibitory effect of the NPY.

As regards M product, the experiment demonstrates that it as low solubility in water, similar to xanthone and coumarin, which confers on these products a lipophilic property; that is, a very good affinity with respect to fats and very good stability.

PATENT CLAIMS

1) Use of a cosmetic composition containing at least one substance that contains a pyrone group and characterized by the fact that it stimulates lipolysis.

2) Use of a cosmetic composition in accordance with Claim 1 and characterized by the fact that activates in the adipocytes phosphorylation of a lipase type enzyme for the breakdown of triglycerides into fatty acids.

3) Use of a cosmetic composition in accordance with Claims 1 or 2 and characterized by the fact that it increases the concentration of cyclic adenosine monophosphate within the adipocytes.

4) Use of a cosmetic composition in accordance with Claim 3 and characterized by the fact that it inhibits a phosphodiesterase enzyme within said adipocytes in such a way that it increases said concentration.

5) Use of a cosmetic composition in accordance with one of Claims 1 to 4 and characterized by the fact that said substance that contains a pyrone group is a molecule [= compound] of the xanthone family of compounds.

6) Use of a cosmetic composition in accordance with Claim 5 and characterized by the fact that said molecule belonging to the xanthone family of compounds is xanthone.

7) Use of a cosmetic composition in accordance with Claim 5 and characterized by the fact that said molecule belonging to the xanthone family of compounds is mangostine.

8) Use of a cosmetic composition in accordance with Claim 7 and characterized by the fact that said mangostine is extracted from the tree of the species *Garcinia mangostana*.

9) Use of a cosmetic composition in accordance with Claim 8 and characterized by the fact that said mangostine is extracted from the sap of said tree.

10) Use of a cosmetic composition in accordance with Claim 8 or 9 and characterized by the fact that said mangostine is extracted from the dried husks and pedicles of the fruit of said tree.

11) Use of a cosmetic composition in accordance with one of the Claims 1 to 4 and characterized by the fact that said substance that contains the pyrone group is composed of a molecule of cojic acid.

12) Use of a cosmetic composition in accordance with one of the Claims 1 to 4 and characterized by the fact that said substance that contains the pyrone group is composed of a molecule from the coumarin family of compounds.

13) Use of a cosmetic composition in accordance with Claim 12 and characterized by the fact that said molecule from the coumarin family of compounds is coumarin.

14) Use of a cosmetic composition in accordance with one of the Claims 1 to 4 and characterized by the fact that said substance that contains the pyrone group is composed of a molecule from the flavenol family of compounds.

15) Use of a cosmetic composition in accordance with Claim 12 and characterized by the fact that said molecule from the flavenol family of compounds is kaempferol.